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Treatment of Petroleum Hydrocarbon Polluted Environment Through Bioremediation: A Review

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Abstract: Bioremediation play key role in the treatment of petroleum hydrocarbon contaminated environment. Exposure of petroleum hydrocarbon into the environment occurs either due to human activities or accidentally and cause environmental pollution. Petroleum hydrocarbon cause many toxic compounds which are potent immunotoxicants and carcinogenic to human being. Remedial methods for the treatment of petroleum contaminated environment include various physiochemical and biological methods. Due to the negative consequences caused by the physiochemical methods, the bioremediation technology is widely adapted and considered as one of the best technology for the treatment of petroleum contaminated environment. Bioremediation utilizes the natural ability of microorganism to degrade the hazardous compound into simpler and non hazardous form. This paper provides a review on the role of bioremediation in the treatment of petroleum contaminated environment, discuss various hazardous effects of petroleum hydrocarbon, various factors influencing biodegradation, role of various enzymes in biodegradation and genetic engineering in bioremediation.

Key words: Bioremediation, petroleum hydrocarbon, environmental contamination, biodegradation

INTRODUCTION

The word petroleum is derived from a Latin word Petraoleum which means rock-oil. Petroleum (liquid)/oil consist of a mixture of liquid hydrocarbon compounds, these hydrocarbon compounds are made up of hydrogen and carbon in different proportions some other elements like sulphur, oxygen and nitrogen are also present in trace amount. In the refining industry, petroleum refers only to crude oil (Van Dyke, 1997). Crude oil is formed in naturally occurring geological deposits formed from organic decomposition products of ancient plant and animal under high temperature and pressure. These petroleum hydrocarbons are the major sources of surface and groundwater contamination. There are many ways of petroleum hydrocarbon contamination some of which includes pipelines and oil wells leakages, wrong methods of disposal of petroleum wastes and accidental oil spills (Amro, 2004). The contamination caused by petroleum hydrocarbon leads to various carcinogenic and neurotoxic effects. Therefore to reduce the hazardous effect of petroleum hydrocarbon, their control and treatment strategies are required. Introducing an extremely important Bioremediation technique to recover contaminated area as compare to other cleaning-up methods with high efficiency and low cost (Bidoia *et al*., 2010). Bioremediation is an eco-friendly process that utilizes naturally occurring microorganisms which transform toxic substances to nontoxic compounds and is emerging as a promising technology for the treatment of soil and groundwater contamination (Milic *et al*., 2009). This review contain an overview of role of bioremediation in petroleum hydrocarbon contaminated environment.

Environmental threats of petroleum hydrocarbon contamination: Petroleum hydrocarbon contamination is highly hazardous to the environment. It has severe impacts on the plant as well as animal ecosystem including human health (Mandal *et al*., 2007). The hazardous effects of petroleum hydrocarbon on various parameters are summarized in Table 1.

Bioremediation: Petroleum spills and their accumulation at refineries, oil distribution pipes, oil storage areas, bus stands, railway stations, airports, gasoline service station, military bases, improper waste disposal methods, industrialization and high standard of living cause a major environmental problem. Thus the clean-up techniques for the contaminated sites are required. Various conventional methods include physical and chemical treatment which

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Table 1: Represents hazardous effects of petroleum hydrocarbon on environment

Parameters	Hazardous effect	References
Agriculture	Soil fertility reduces, physiological properties of soil affects, adverse effect on seed germination,	Yoshida *et al.* (2006), Gong *et al.* (2001), Wyszkowska and Kucharski (2000), De Jong (1980)
Aquatic life	Death of natural flora and fauna (oil spill cause anaerobic condition), aquatic birds suffer from Hypothermia, Drowning, loss in flight, Poisoning, reproductive Impairment in fish	Carneiro *et al.* (2010), Torres *et al.* (2008), Gelin *et al.* (2003), Peterson (2001), Wrabel and Peckol (2000)
Human	Severe diseases (skin erythema (reddening), skin cancer, sinonasal cancer, gastrointestinal cancer and bladder cancer), effect on CNS, depression, irregular heartbeats	Lewis *et al.* (2008), Chen *et al.* (2008), Rice *et al.* (2007), Lee *et al.* (2006), Knafla *et al.*(2006), Gomer and Smith (1980)
Ecosystem	Imbalance in marine ecosystem, Physical and chemical alteration of natural habitats, imbalance in food chain.	Carneiro *et al.* (2010), Torres *et al.* (2008), Gelin *et al.* (2003), Peterson (2001), Wrabel and Peckol (2000)
Plants	Plants covered by oil are unable to photosynthesize	Szaro *et al.* (1978), Gelin *et al.* (2003), Carneiro *et al.* (2010), Torres *et al.* (2008), Samiullah (1985), Wrabel and Peckol (2000), Peterson (2001)
Animals	Crude oil exposure may cause damage to lungs, liver, kidneys, intestines and other internal organs	Gomer and Smith (1980), Knafla *et al.* (2006), Zhang *et al.* (1992), Lewis *et al.* (2008) and Rice *et al.*(2007)

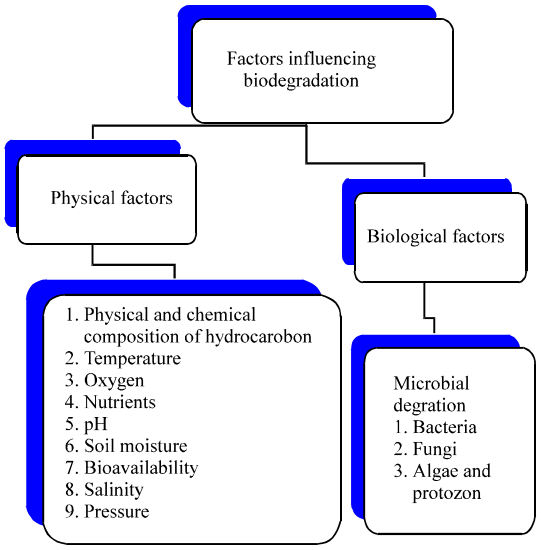

Fig. 1: Represents various factors that influence the rate of biodegradation

are costly and environment effective. Thus introducing a prime candidate for the treatment of petroleum contaminated site are Bioremediation technologies. Bioremediation is a managed or spontaneous process by which environmental contamination can be removed by applying biological remedies especially microbiological, catalysis act on pollutant compounds (Madsen, 1991; Plaza *et al*., 2001). Bioremediation leads to complete mineralization of complex organic pollutants to simple inorganic compounds, e.g., CO_2 and H_2O but conventional methods such as adsorption, vapourization and extraction reduces soil fertility, adversely affect the natural processes in environment and mostly transfer thecontaminant from one environment to another, e.g. soil to the atmosphere (Semple *et al*., 2001; Plaza *et al*., 2001).

Bioremediation is based on the concept of biotransformation and biodegradation, in which the toxic contaminant is transformed or degraded to non-toxic compound. There are two main processes included in bioremediation are biostimulation and bioaugmentation. Biostimulation is the process of addition of nutrients into the contaminated site to increase the growth and metabolic activities of the indigenous microbes for the degradation of pollutants. Bioaugmentation is the process of addition of specific microorganisms or genetically engineered microorgamisms into the contaminated site for the degradation of specific pollutant and also to make degradation process faster and better. To speed up bioremediation, we must provide an ideal growing environment with proper temperature, pressure, nutrients, moisture, oxygen and pH. Bioremediation stands to benefit greatly and advances even more rapidly with the adoption of recombinant DNA technology. In order to make bioremediation successful, the choice of appropriate microorganisms is essential for the degradation of pollutant at the contaminated site under favorable environmental conditions.

Factors influencing biodegradation of petroleum hydrocarbon: There are various factors which are identified as limiting factors for the biodegradation of petroleum hydrocarbons. To make bioremediation successful, the proper knowledge of environmental parameters that influence the biodegradation of contaminant is necessary. The various factors that influence the rate of biodegradation are represented through Fig. 1.

Physical factors: The various physical parameters that affect the rate of biodegradation are summarized in the Table 2.

Table 2: Represents various physical factors that affects rate of biodegradation

Parameter	Condition	Reference
Structure and composition of hydrocarbon	Hydrocarbon susceptibility to microbial attack is in the order of: n-alkanes >branched alkane>low molecular weight aromatics>cyclic alkanes.	Perry (1984)
	Or Biodegradation rate: Saturates> light aromatics>high molecular weight aromatic>polar compounds.	Fusey and Oudot (1984), Jobson *et al.* (1972), Walker *et al.* (1976)
Temperature	At low temperature, the viscosity of oil increases while volatility of toxic low molecular weight hydrocarbons reduced, decreasing biodegradation.	Atlas (1975)
	With decrease in temperature the rate of degradation also decreases because of decreasing enzymatic activity.	Atlas and Bartha (1972), Gibbs *et al.* (1975)
	Highest degradation rate occur in the range : 30-40°C (soil), 20-30°C(fresh water), 15-20°C(marine).	Bartha and Bossert (1984), Cooney (1984)
	Significant biodegradation of hydrocarbon reported in psychrophilic environment in temperate region.	Yumoto *et al.* (2002), Pelletier *et al.* (2004), Delille *et al.* (2004)
Oxygen	Catabolism of aliphatic, cyclic and aromatic hydrocarbon by bacteria and fungi in the initial step involves the oxidation of the substrate by oxygenases, for which molecular oxygen is required.	Singer and Finnerty (1984), Perry (1984), Cerniglia (1984a, b)
	The concentration of oxygen has been identified as the rate limiting variable in the biodegradation of petroleum in soil.	Von Wedel *et al.* (1988)
	The oxygen availability in soil depends on rates of O_2 consnmption by microbes, the type of soil and presence of utilizable substrates, which lead to oxygen depletion.	Bossert and Bartha (1984)
Nutrients	Addition of nutrients is necessary to enhance the biodegradation of oil pollution.	Choi *et al.* (2002), Kim *et al.* (2005)
	Excessive nutrient concentration can also inhibit the biodegradation activity. A negative effect of high NPK levels on the biodegradation of hydrocarbons has been reported.	Chaillan *et al.* (2006)
	Poultry manure used as organic fertilizer in contaminated soil enhanced the rate of biodegradation.	Oudot *et al.* (1998), Chaineau *et al.* (2005), Carmichael and Pfaender (1997)
	Photo-oxidation increased the biodegradability of petrolenm hydrocarbon by increasing its bioavailability and thus enhancing microbial activities.	Okolo *et al.* (2005), Maki *et al.* (2001)
Acidity and alkalinity	The pH of soil is highly variable: Mine spoils-2.5 Alkaline desert-11.0.	Bossert and Bartha (1984)
	A pH neutral is favoured by most heterotrophic bacteria and fungi.	Atlas (1988)
Soil moisture	Water potential(a_w) of soils can range from 0.0-0.99, aquatic environment-0.98.	Bossert and Bartha (1984)
	Optimal rates of biodegradation of oil sludge in soil, at 30 to 90% water saturation.	Dibble and Bartha (1979)
Bioavailability	The application of external non ionic surfactant (oil spill dispersants) influences the alkane degradation rate.	Bruheim and Eimhjellen (1998), Rahman *et al.* (2003)
	Application of surfactants in oil contaminated area may have a simulatory, inhibitory or neutral effect on the bacterial degradation of the oil compound.	Liu *et al.* (1995) Van Dyke *et al.* (1991),
	Biosurfactants and biosurfactants-producing bacteria have been investigated for their ability to increase bioavailability.	Volkering *et al.* (1993) Miller (1994)
Salinity	The rate of hydrocarbon metabolism decreased with increasing salinity in the range 3.3 to 28.4% and attributed the results to a general reduction in microbial metabolic rates.	Ward and Brock (1978)
Pressure	Degradation of hydrocarbon substrate by a mixed culture of deep-sea sediment bacteria was monitored at 10 atm and 495 or 500 atm.	Schwarz *et al.* (1974), Schwarz *et al.* (1975), Schwarz *et al.* (1974)

Table 3: Represents various microorganisms that degrade petrolenm hydrocarbon

	Microorganism	Reference
Bacteria	*Pseudomonas*, *Micrococcus* and *Nocardia* spp.	Austin *et al.* (1977)
	Pseudomonas putida	Prescott *et al.* (2002)
Fungi	*Amorphoteca*, *Neosartoya*, *Talaromyces* and *Graphium*	Chaillan *et al.* (2004)
	Aspergillus, *Cephalosporium* and *Penicillium*	Singh (2006)
Yeast	*Candida*, *Yarrowia*, *Pichia*	
	Candida lipolytica, *Rhodotorula mucilaginosa*, *Geotrichum* sp.,	Chaillan *et al.* (2004)
	Trichosporon mucoides	Boguslawska-Was and Dabrowski (2001)
Algae	*Prototheca zopfi*	Walker *et al.* (1975)

Biological factors: Biological factors include various microorganisms that play important role in the degradation of petroleum hydrocarbon. The various microorganisms are listed in Table 3.

Enzymes in bioremediation: Degradation of petroleum components by microorganisms is possible due to the presence of enzymes secreted by them. The complex hydrocarbons can be degraded by those microorganisms which possesses high enzymatic activity. Alexander (1994). Chromosomal or plasmid DNA is the sites where the gene locates which is responsible for the production of enzyme required in the degradation of petroleum hydrocarbon (Broderick, 1999). The aerobic and anaerobic both situations lead to degradation of petroleum hydrocarbons (Van Hamme *et al.*, 2003). When

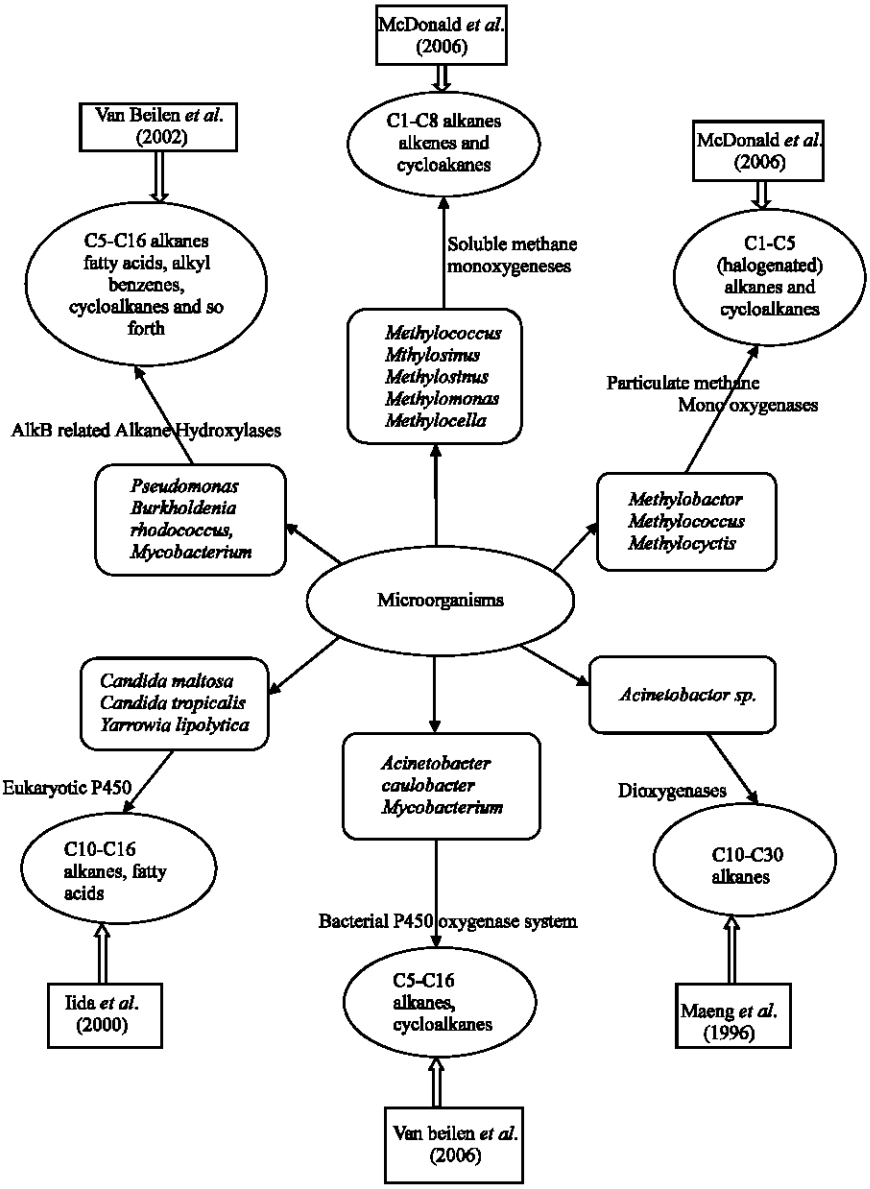

Fig. 2: Listed some hydrocarbon degrading fungi

degradation occurs in aerobic condition, the enzyme oxygenase is responsible for the introduction of oxygen atom into the hydrocarbons and when degradation occurs in anaerobic condition, the degradation is catalysed by anaerobic bacteria for example Sulphate-reducing bacteria, employing various terminal electron acceptors. For successful bioremediation, enzymes play important role and it depends on the capability of enzyme to degrade or detoxify the complex pollutant such as petroleum into the simpler or non toxic form. Figure 2 shows certain enzymes produced by some microorganisms to degrade hydrocarbons of different size/length.

Biotechnological aspects in bioremediation: Biotechnological aspects in bioremediation involve the application of Genetically Engineered Microorganisms (GEMs). These genetically engineered microorganisms hold tremendous potential in the treatment of environmental pollution. Genetic engineering techniques offer the possibility to equip organisms, known to survive and be active when introduced into certain environments, with the desired catabolic potential (Halden *et al*., 1999). Genetic engineered organisms play important role in biodegradation of polyaromatic hydrocarbons (PAHs), which occur in soil due to spills or leakage of petroleum

products. The first genetically engineered organism for bioremediation was *Pseudomonas*, introduced by Dr. Anand Mohan Chakrabarty in USA and proved to be effective in treating environment contaminated with petroleum hydrocarbon. Recombinant DNA technology may also allow the construction of bacterial and fungal strains exhibiting improved capability for hydrocarbon metabolism and suitability as seed organisms for the elimination of hydrocarbon pollutants (Leahy and Colwell, 1990). Priority area of research on Recombinant DNA technology/genetic engineering in Bioremediation includes (Pieper and Reineke, 2000):

- Improving efficiency of microbial strains
- Improving efficiency of bioanalytical methods for measuring the level of contaminants
- Developing analytical techniques for better understanding, control and optimization of environmental and reactor system
- Characterizing the microbial community structures and activities to predict the performance of microorganisms under in situ conditions and in concert with the indigenous microflora

Why using bioremediation as a tool in the petroleum hydrocarbon contaminated environment:

- Bioremediation is an environment friendly approach and is therefore accepted by the public as a remedy in the treatment of contaminants
- Bioremediation is less expensive as compared with other conventional techniques (land filling or incineration)
- Bioremediation can be done on site and site disruption is minimal
- It eliminates waste and also eliminates the chance of future liability associated with treatment and disposal of contaminated material
- The microorganisms involved in the degradation of contaminant increases in their number till the contaminant is present. After the degradation of contaminant the microbial population itself decreases naturally
- Bioremediation transforms the toxic substances to harmless products such as CO_2 (utilized by plants in photosynthesis), H_2O and fatty acids

CONCLUSION

The application of bioremediation technology in the environment contaminated with petroleum hydrocarbon is the major issue in current scenario. In the developed countries, the amounts of petroleum stocks are enormous and petroleum transportation across the world is frequently thus, possibilities of oil spills increases. Bioremediation play important role in the environment contaminated with petroleum hydrocarbon. Various physical and chemical methods used for the treatment of petroleum contaminated area cause negative impact on environment and are also very costly. Therefore because of lower cost, easy recovery of contaminated land in short period and eco-friendness, bioremediation is used as one of best technology in the remediation of petroleum contaminated environment. In spite of all this, still a research is needed in the field of bioremediation for designing and development of the bioremediation technologies that are appropriate for site with complex mixture of contaminants. More concentration is required in the treatment part of bioremediation. A pilot study should be performed before applying this technique in the large scale. Pilot study should be done to check various environmental conditions are appropriate for degradation or not, the contaminant is biodegradable or not, what steps should be taken if contaminant is non biodegradable. In an environment the fate of petroleum hydrocarbon is important to evaluate the environmental threat of oil spills and to develop biotechnology to cope with them.

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